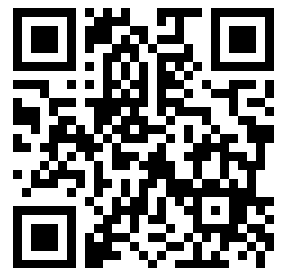


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**THE EFFECT OF X IRRADIATION UPON THE PERFORMANCE  
OF VOLITIONAL ACTIVITY BY THE ADULT MALE RAT**

**D. C. Jones  
D. J. Kimeldorf  
D. O. Rubadeau  
G. K. Osborn  
T. J. Castanera**

**USNRDL-400**

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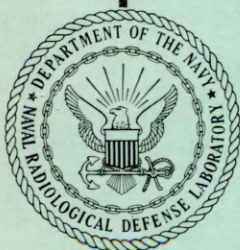
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THE EFFECT OF X IRRADIATION UPON THE PERFORMANCE  
OF VOLITIONAL ACTIVITY BY THE ADULT MALE RAT

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USNRDL-400  
Health and Biology

Technical Objective  
AW-6

Physiology-Psychology Branch  
D. J. Kimeldorf, Head

Biological and Medical Sciences Division  
M. C. Fishler, Head

Scientific Director  
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Director  
Captain J. L. Bird, USN

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U. S. NAVAL RADIOLOGICAL DEFENSE LABORATORY  
San Francisco 24, California

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17 April 1953

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U N C L A S S I F I E D

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**ABSTRACT****USNRDL-400**

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Standard volitional activity wheels were used to determine the effects of single acute doses of X rays upon adult male Sprague-Dawley rats in a study of physical performance and malaise. Under controlled conditions of light, temperature, and sound level, the daily activity of each animal was recorded for consecutive periods as long as 11 weeks after X-ray doses of 200, 300, 400, 540, 680, 800, 900, or 1,000 r.

At all doses studied there was an abrupt significant depression of activity. This initial decrease in activity was immediately followed by a period of recovery (increasing activity). At 200 and 300 r recovery was complete within 5 days of irradiation and there was no further discernable effect of irradiation at this level upon volitional activity. At doses of 400 r or greater, there was a second significant decrease in activity which reached a minimum value during the third week after irradiation. The time necessary for survivors to complete recovery from this second decrease in activity appeared to be proportional to the X-ray dose. When animals died within the first 9 days after irradiation, their activity decreased continuously from the time of irradiation until death. With decedents which survived longer than 9 days, the initial decrease in activity was followed by some recovery and then by a second decrease in activity which continued until death.

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INTRODUCTION

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USNRDL-400

Since its introduction by Stewart in 1898,<sup>1</sup> the volitional activity wheel apparatus has been used by numerous investigators to measure spontaneous activity in the rat. A comprehensive review of the field prior to 1929 was made by Shirley,<sup>2</sup> and a similar review covering the period from 1929 to 1947 was made by Reed.<sup>3</sup> In recent years the wheel apparatus has been used to measure volitional activity in studies of electroconvulsive shock,<sup>4,5</sup> nutrition,<sup>6-9</sup> and genetics,<sup>10</sup> and to assay the effect of low doses of X rays delivered to the hypophysis upon the estrus cycle in rats.<sup>11</sup>

In studying the effects of whole-body ionizing radiations upon the intact animal, the volitional activity wheel was selected as one means of evaluating the interrelationships between irradiation and physical performance. Investigators studying the effects of ionizing radiations have long been aware that the irradiated animal frequently appears to be in a state of general "poor health". Such signs as ruffled fur, encrustations about the muzzle and eyes, loss of weight, diarrhea, decreased food consumption, and relative inactivity are all commonly observed in animals subjected to appropriate amounts of ionizing radiations. One can reasonably assume that in addition to such readily observable signs of physiological disturbances, the irradiated animal probably does not "feel well", or, to employ the term used in humans, is suffering from malaise. The volitional activity wheel was chosen in an attempt to evaluate this altered psycho-physiological state in animals. It was felt that this apparatus might provide a means of obtaining a quantitative index of malaise, since operation of the wheel involves not only the ability but the motivation of the animal to engage in this particular form of activity. Obviously, in considering the results of such a study one must always keep in mind the fact that such a measure is the result of a combination of both motivation and ability. The present study includes the effects of single acute doses of X rays from 200 to 1,000 r upon volitional activity.

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**EXPERIMENTAL PROCEDURES**

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USNRDL-400

One hundred and ninety-four male Sprague-Dawley rats  $72 \pm 3$  days of age, of inbred USNRDL stock, were used in this study. The volitional activity of each animal was recorded daily for periods of observation as long as 13 weeks. Animals were maintained on a stock diet of Purina Laboratory Chow which had been ground to a meal in order to prevent jamming of the wheel cage by food pellets.

The experimental apparatus was a standard activity wheel, having a drum  $4\frac{1}{2}$  in. wide and 14 in. in diameter. Each wheel was equipped with a Veeder-type counter which recorded revolutions of the wheel in both directions. Food and water were placed in an attached living cage, 10 by 6 by 5 in. An opening in the living cage provided free access to the wheel. Prior to each series, wheels were calibrated using the system recommended by Lacey.<sup>12</sup> It was found that the distance through which the animal must move along the wheel in order to overcome starting friction was less than 0.4 cm for each cage. Each wheel cage apparatus was placed in a sound-damped box which was open at the front. The living cage faced the open side of the box, thus providing maximum sound and visual isolation of the activity wheel. Up to 12 such boxes were placed in each of ten sound-proofed rooms. All sound insulation was accomplished through the use of  $1\frac{1}{4}$  in. acousti-celotex lining. Each room was illuminated constantly by a single 150 watt incandescent bulb, and each was supplied with its own air exchange system. The concrete building containing the ten rooms was equipped with a thermostatically controlled steam heat system. Mean daily temperatures within the rooms ranged from 74 to 81 F for the 540 and 680 r animal groups; 70 to 75 F for the 800, 900, and 1,000 r animal groups; and 69 to 75 F for the 200, 300, and 400 r animal groups.

Each experiment commenced on a Monday, and wheel readings were taken daily at the same time. Feeding and watering were accomplished between 1000 and 1100 each Friday, and cleaning occurred at the same time on alternate weeks. The animals were not otherwise disturbed, except for irradiation or sham irradiation.

The radiation factors were as follows: X rays, 250 KVP; 15 ma; filter, 0.5 mm Cu + 1.0 mm Al (HVL 1.5 mm Cu); TSD, 40 in.; and 25 r per min (air dose), measured with Victoreen thimble chambers. For exposure to the X rays, animals were placed in Lucite chambers constructed to approximate an isodose surface. The chambers were



spaced radially on a motor-driven turntable which revolved slowly in the radiation field. Nonirradiated controls were confined in the irradiation chambers for the same lengths of time as the irradiated animals. Irradiation occurred immediately after the fourteenth 24 hr period in the apparatus. The 24 hr period following irradiation was designated as day one post-irradiation. The total X-ray doses (air) were 200, 300, 400, 540, 680, 800, 900, and 1,000 r.

The study was comprised of three experiments, each having a separate nonirradiated control group. The first (Experiment A) included the 540 and 680 r groups; the second (Experiment B) the 800, 900, and 1,000 r groups; and the third (Experiment C) the 200, 300, and 400 r groups. In considering the results of this study, each of the eight irradiated groups was compared with the appropriate control group which was sham irradiated in similar containers.

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## RESULTS

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Two methods of grouping the large number of individual daily activity values were selected. During the 2 weeks prior to irradiation all of the animals were experimentally similar, and differences among groups were presumably the result only of the usual variations among individuals. Activity during this period is summarized as the weekly mean revolutions per 24 hr for each group, as shown in Tables 1, 2, and 3. Following irradiation it was observed that appreciable changes in activity were occurring on a day to day basis. In order to illustrate these changes and simultaneously to discern trends, activity during this period is expressed as the moving 3 day average\* of the daily mean revolutions for each group. Depending upon the X-ray dose, the day to day change in response was observed to continue for periods of time from a few days (200, 300 r) up to 5 weeks (680 r) after irradiation. For uniformity, the 3 day moving average is used for all groups during the first 5 weeks after irradiation. Throughout the remainder of the experimental period only gradual changes in activity were apparent, and daily readings for this period are summarized as the weekly mean revolutions per 24 hr for each group. The median test described by

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\* The average of the group mean revolutions for each three adjacent days, i. e., days 1-2-3, 2-3-4, 3-4-5, etc.<sup>13</sup>



Moses<sup>14</sup> was used for statistical analysis of the observed differences between experimental and control groups with respect to the average of any 3 consecutive days. Since the composition of a group changes from time to time when nonsurviving animals are included with survivors, separate data are presented for animals surviving the irradiation and for those which succumbed. Mortality data are summarized in Table 4.

### Controls

Activity levels in terms of revolutions are presented in Tables 1, 2, and 3, and the differences in revolutions between irradiated and control groups are illustrated in Figs. 1 and 2. As is usual in activity studies of this nature, the daily standard deviations within groups were found to be of the order of 30 per cent of the mean activity. It has been previously reported that the activity level of normal animals changes with age and experience in the wheel apparatus.<sup>15</sup> Therefore, in describing the activity patterns of animal groups, the data are discussed in terms of changes with time in the activity of a given group and in terms of the activity of an irradiated group relative to that of the appropriate control group.

The general pattern of activity with time was similar for control groups among the three experiments. All animals were similar with respect to genetic constitution and age, and were subjected to identical experimental procedures, other than irradiation dose. There were some differences in environmental temperature and sound level resulting from the chronological sequence of experiments. These factors may be responsible for the small differences in the detailed appearance of the time course of the control activity level among the three experiments. Examination of Tables 1 and 2 shows that the degree of change in activity following sham irradiation varied somewhat among the three groups of controls. This variation may have been due to the use of slightly less confining irradiation containers in the latter two experiments and to differences in duration of confinement, since each control group was confined for a period of time equivalent to that required for irradiation of the concurrent irradiated group receiving the highest dose.

### Volitional Activity of Surviving Irradiated Animals

All irradiated animal groups exhibited an immediate depression in activity level following irradiation, both in absolute (Table 1) and relative (Fig. 1) terms. The extent of this depression appeared to be

generally greater with increasing dose, and the difference in activity level between irradiated and control groups was significant ( $p < 0.05$ ) at all doses immediately after irradiation (Table 1). Activity levels for all irradiated groups then increased rapidly and this increasing activity (recovery) persisted longer in the 540 and 680 r groups than in the 200, 300, and 400 r groups (Table 1). The extent of this relative recovery was generally similar at 200, 300, and 400 r; less at 540 r; and least at 680 r (Fig. 1). The latter group exhibited a continued relative decrease in activity for several days, and relative recovery commenced later and continued for a shorter period than at the lower doses. It is of interest that despite the recovery shown by the 680 r group, significant differences from control activity continued throughout this period (Table 1).

At the three higher doses (400, 540, 680 r) there was a second decrease in activity, starting within a few days after the initial recovery phase and continuing for about 8 to 10 days (Table 1). The extent of this second depression in activity was generally proportional to the X-ray dose. Significant differences from control activity were apparent in the 400 and 540 r groups during the latter part of this phase, while the 680 r group continued to exhibit a significant difference from controls (Table 1).

All three of these groups then showed rapid recovery from this second depression in activity (Table 1). The 400 and 540 r groups attained levels of activity in the range of control activity about 4 weeks after irradiation (Fig. 1). The 680 r group was somewhat slower in recovery and did not attain an activity level in the range of that of controls until about 8 weeks after irradiation, although there were no statistically significant differences from control activity after about one month post-irradiation. No further appreciable differences in activity among the irradiated and control animals were evident.

#### Volitional Activity of Nonsurviving Irradiated Animals

Data for nonsurviving animals were divided into two categories according to post-irradiation survival time. At all doses studied for those animals which succumbed to irradiation within 9 days activity levels decreased rapidly until death (Table 2 and Fig. 2). The three animals of the 680 r group in this category exhibited a slightly lower rate of decrease than animal groups at 800, 900, and 1,000 r, but all reached similar minimum activity levels prior to death. However, for those animals surviving longer than 9 days after irradiation, activity levels increased after an initial post-irradiation depression and then dropped rapidly prior to death (Table 3, Fig. 2). The extent of the initial decrease, but not of recovery, appeared to be proportional

to dose. The extent of the second decrease in activity prior to death appeared to be inversely proportional to dose, so that similar terminal activity levels were evident for all doses.

In all survivor groups, and in nonsurvivors at 680 r, volitional activity began to increase rapidly immediately after the initial post-irradiation decrease (Tables 1, 2, and 3). This would seem to emphasize the fact that at sublethal or low lethal doses one is confronted with a recovering animal in terms of volitional activity, beginning very soon after irradiation. Even at higher doses (800, 900 r) it appears that in those cases where the animal survives the first 9 days, some degree of recovery in activity is possible during the first week after irradiation.

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## DISCUSSION

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The relationship of the current study to the literature in the field of radiation biology is difficult since no previous studies of radiation injury using volitional activity as a criterion are known to the authors. In attempting to evaluate the significance of the observed changes in volitional activity one approach is to determine what factors known to affect volitional activity are also known to be altered by irradiation. Dietary intake appears to fulfill both requirements. However, examination of the results of studies of changes in body weight<sup>16,17</sup> and of food<sup>17</sup> and water<sup>16</sup> consumption after irradiation indicates that there is a relatively small degree of similarity between the time sequence of these criteria of radiation damage and that of changes in volitional activity. Recovery from the initial decrease in volitional activity appears to occur prior to the time of recovery from the reported initial decreases in body weight and food consumption, and the second decrease in volitional activity found at 400 and 540 r apparently has no counterpart in food intake or body weight changes at equivalent doses. The second decrease in volitional activity of nonsurvivors which survived beyond the ninth day commences somewhat sooner than the second decrease in body weight and food consumption found in nonsurvivors which survived beyond the first week.<sup>17</sup> Volitional activity of nonsurvivors which died within the first 9 days decreased until death, as did body weight and food intake in comparable animals in the above investigations;<sup>16,17</sup> however, this does not necessarily imply any causal relationship. No consistent

similarities between changes in volitional activity and water consumption are apparent since polydipsia was found at all doses on days 1 and 2, and again on day 5 in survivors of lethal doses by Smith, et al.,<sup>17</sup> and by Nims and Sutton<sup>16</sup> on day 1 and again after a latent period of 1 to 4 days depending upon dose.

Previous studies of the effect of irradiation on the ability of rats to perform daily intensive exercise to exhaustion<sup>18</sup> have indicated that there is a gradual decrease in the ability to perform this type of exercise for 4 or 5 weeks after irradiation, followed by recovery. There appears to be no similarity between the time pattern of changes in exhaustive exercise ability and the sequence of changes in volitional activity levels resulting from irradiation. Since rats are apparently quite capable of performing exhaustive exercise immediately after irradiation, it appears possible that the initial depression in volitional activity may be related to a decreased motivation for exercise rather than to a lowered ability.

While no extrapolation from the conclusions of this study to the prediction of phenomena of radiation injury in humans is intended or implied, it is of interest to note that there appears to be a remarkable degree of similarity between the time course of changes in the volitional activity of animals after irradiation and some of the phenomena reported to occur in humans exposed to acute whole-body ionizing radiations. In reports concerned with the medical effects of atomic bombs,<sup>19,20,21</sup> the number of people exhibiting malaise and vomiting on the day of bombing was considered remarkably large, and after a period of relative well-being which lasted from several days to a few weeks, a phase of fever and hemorrhage was also noted. In a review suggesting clinical procedures to be employed in the event of radiation injuries produced by atomic bombs, Cronkite<sup>22</sup> noted that persons receiving supralethal doses of ionizing radiations would probably vomit continuously beginning within a few hours of exposure, followed by prostration and death within a few days. In the current study, animals which died within the first 9 days (680 to 1,000 r) exhibited an essentially continuous decrease in activity, suggesting increasing malaise during this period. It was suggested that, in persons receiving doses of ionizing radiations in the lethal range, vomiting would probably occur on the day of bombing and subside within 24 hr, followed by recrudescence of symptoms in 1 to 3 weeks.<sup>22</sup> In the present study those animals irradiated with 400 or 540 r and those animals which survived more than 9 days after a dose of X rays in the lethal range (680 r) exhibited decreased activity on the day of irradiation followed by recovery and then by a second decrease in activity beginning in about 10 days. For humans Cronkite predicted prostration and death within a few days following irradiation with supralethal doses. In the present study those animals which survived more than 9 days after X-ray doses of 800 and 900 r exhibited

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some degree of recovery prior to death. For those persons receiving sublethal doses of irradiation, Cronkite predicted no vomiting on the day of irradiation and milder, if any, late symptoms similar to those in persons receiving doses in the lethal range. In the present study, volitional activity levels were depressed significantly on the day of irradiation at 200 and 300 r, although no second decrease in activity was apparent. At higher doses in the sublethal range (400 and 540 r) a second decrease in activity occurred although to a lesser extent than in the lethal range (680 r).

Perhaps the most thoroughly documented report of the acute irradiation syndrome in man is the one concerned with the medical effects of the accidental exposure of nine persons at the Los Alamos Scientific Laboratory.<sup>23</sup> Although there was more than one type of radiation, the doses were calculated in terms of roentgen equivalents of 80 kv X rays and gamma rays. Of the 3 cases receiving the equivalent of about 400 r or greater, all exhibited vomiting on the day of exposure, felt better for the next 3 or 4 days (in spite of the fact that 2 of the 3 ultimately died), and then entered a febrile phase beginning on the sixth day after exposure. One more of the remaining six cases, who received an estimated dose of about 45 r, was nauseated on day one, but this might have been largely a psycho-physiological reaction to an emotionally traumatic event. Thus it appears—assuming nausea and vomiting are accompanied by a general feeling of illness—that the time pattern of malaise in humans exposed to acute ionizing radiation is not unlike the sequence of changes in volitional activity induced in animals by exposure to acute doses of X rays.

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**SUMMARY**

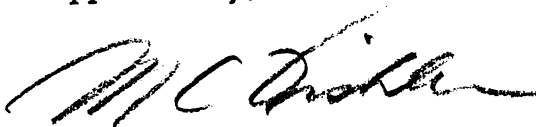
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**USNRDL-400**

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The effect of single acute doses of X rays, ranging from 200 to 1,000 r, upon the daily volitional activity level of adult male rats was determined in a study of malaise and physical performance. At all doses studied there was an abrupt significant depression of activity. This initial decrease in activity was immediately followed by a period of recovery (increasing activity). At 200 and 300 r recovery was complete within 5 days of irradiation and there was no further discernible effect of irradiation at this level upon volitional activity. At doses of 400 r or greater, there was a second significant decrease in activity which reached a minimum value during the third week after irradiation. The time necessary for survivors to complete recovery from this second decrease in activity appeared to be proportional to the X-ray dose. When animals died within the first 9 days after irradiation, their activity decreased continuously from the time of irradiation until death. With decedents which survived longer than 9 days, the initial decrease in activity was followed by some recovery and then by a second decrease in activity which continued until death.

Approved by:



M. C. FISHLER, Head  
Biological and Medical  
Sciences Division

For the Scientific Director

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TABLE 1

Volitional Activity of Survivors Expressed in Mean Revolutions per 24 Hr

Observation Period		Mean Revolutions <sup>(a)</sup>						
		Experiment C				Experiment A		
		Control (n = 26)	200 r (n = 27)	300 r (n = 23)	400 r (n = 25)	Control (n = 15)	540 r (n = 17)	680 r (n = 7)
Preirradiation								
Week 1		323	326	271	257	354	323	341
	2	623	599	576	583	743	719	761
Post-irradiation								
Trial Days	1- 3	705	*411	*391	*376	533	*209	* 96
	2- 4	823	591	564	*552	667	*350	*130
	3- 5	800	659	652	633	725	*426	*134
	4- 6	762	677	684	678	824	*540	*207
	5- 7	762	678	693	705	829	626	*291
	6- 8	753	659	667	704	866	697	*375
	7- 9	762	655	672	710	863	709	*411
	8-10	759	654	667	681	921	734	*406
	9-11	759	648	638	634	880	736	*361
	10-12	716	640	606	601	844	676	*303
	11-13	725	661	615	599	841	599	*245
	12-14	765	689	660	612	871	535	*200
	13-15	830	728	691	*617	863	*523	*156
	14-16	844	729	710	*617	835	*486	*116
	15-17	841	732	710	*624	863	*482	* 91
	16-18	811	700	692	593	878	*492	* 79
	17-19	750	665	644	554	837	*460	* 65
	18-20	739	651	637	544	794	*452	* 56
	19-21	738	670	638	568	784	475	* 66
	20-22	773	698	663	606	865	534	* 97
	21-23	747	690	649	620	838	544	*132
	22-24	766	676	643	631	779	545	*166
	23-25	723	644	615	620	661	540	*193
	24-26	715	637	583	624	556	489	*211
	25-27	697	634	587	642	533	466	*234
	26-28	766	691	631	690	547	452	*270
	27-29	789	715	660	701	631	520	*330
	28-30	773	721	657	672	632	526	*350
	29-31	732	677	630	628	616	528	*375
	30-32	683	608	614	574	658	595	*390
	31-33	627	552	575	539	650	589	419
	32-34	641	562	582	566	672	607	428
	33-35	669	616	612	617	674	601	456
Week 6		666	614	576	624	646	685	452
	7	679	578	592	606	540	578	435
	8	625	536	564	571	575	609	485
	9	573	518	558	583	566	592	491
	10	554	492	536	564	537	570	478
	11	555	481	520	513	506	560	487

(a) The letter n signifies the number of animals in each group. Points differing significantly ( $p < 0.05$ ) from control activity, using the median test described by Moses,<sup>13</sup> are indicated by asterisk.

TABLE 2

The Volitional Activity of Nonsurviving Animals  
Which Died prior to the Tenth Day after Irradiation  
Expressed in Mean Revolutions per 24 Hr

Observation Period	Mean Revolutions <sup>(a)</sup>					
	Experiment A		Experiment B			
	Control (n = 15)	680 r (n = 3)	Control (n = 12)	800 r (n = 2)	900 r (n = 3)	1,000 r (n = 14)
Preirradiation						
Week 1	354	316	349	583	374	397
2	743	795	739	705	724	709
Post-irradiation						
Trial Days 1-3	533	146	754	89	68	75
2-4	667	228	848	72	72	66
3-5	725	252	844	35	29	26
4-6	824	217	869	33	-	19
5-7	829	114	884	10	-	23
6-8	866	14	-	-	-	-
7-9	863	17	-	-	-	-

(a) The letter n signifies the number of animals in each group.

TABLE 3

The Volitional Activity of Nonsurviving Animals  
Which Died Later Than the Ninth Day after Irradiation  
Expressed in Mean Revolutions per 24 Hr

Observation Period		Mean Revolutions <sup>(a)</sup>				
		Experiment A		Experiment B		
		Control (n = 15)	680 r (n = 4)	Control (n = 12)	800 r (n = 9)	900 r (n = 7)
Preirradiation						
	Week 1	354	257	349	397	335
	2	743	558	739	710	641
Post-irradiation						
Trial Days	1- 3	533	144	754	112	92
	2- 4	667	223	848	138	86
	3- 5	725	241	844	123	64
	4- 6	824	399	869	186	70
	5- 7	829	456	884	271	84
	6- 8	866	525	899	339	86
	7- 9	863	525	888	388	115
	8-10	921	541	889	342	125
	9-11	880	432	884	206	88
	10-12	844	277	926	136	69
	11-13	841	131	949	73	-
	12-14	871	110	980	35	-
	13-15	863	31	947	18	-
	14-16	835	17	-	-	-
	15-17	863	12	-	-	-
	16-18	878	-	-	-	-
	17-19	837	-	-	-	-
	18-20	794	-	-	-	-
	19-21	784	-	-	-	-
	20-22	865	-	-	-	-

(a) The letter n signifies the number of animals in each group.

TABLE 4

The Survival Rates for Groups of Animals  
Irradiated with Lethal or Supralethal Doses of X Rays

Time Post-irradiation (days)	Survival Rates							
	680 r		800 r		900 r		1,000 r	
	Fraction	%	Fraction	%	Fraction	%	Fraction	%
1	14/14	100	11/11	100	10/10	100	14/14	100
2								
3								
4								
5			10/11	91	9/10	90	8/14	57
6					7/10	70	2/14	14
7								
8	13/14	93	9/11	82			1/14	7
9	12/14	86			6/10	60	0/14	0
10	11/14	79			5/10	50		
11								
12	10/14	71	6/11	55	1/10	10		
13	9/14	64	3/11	27	0/10	0		
14			2/11	18				
15			1/11	9				
16			0/11	0				
17								
18	7/14	50						
75	7/14	50						

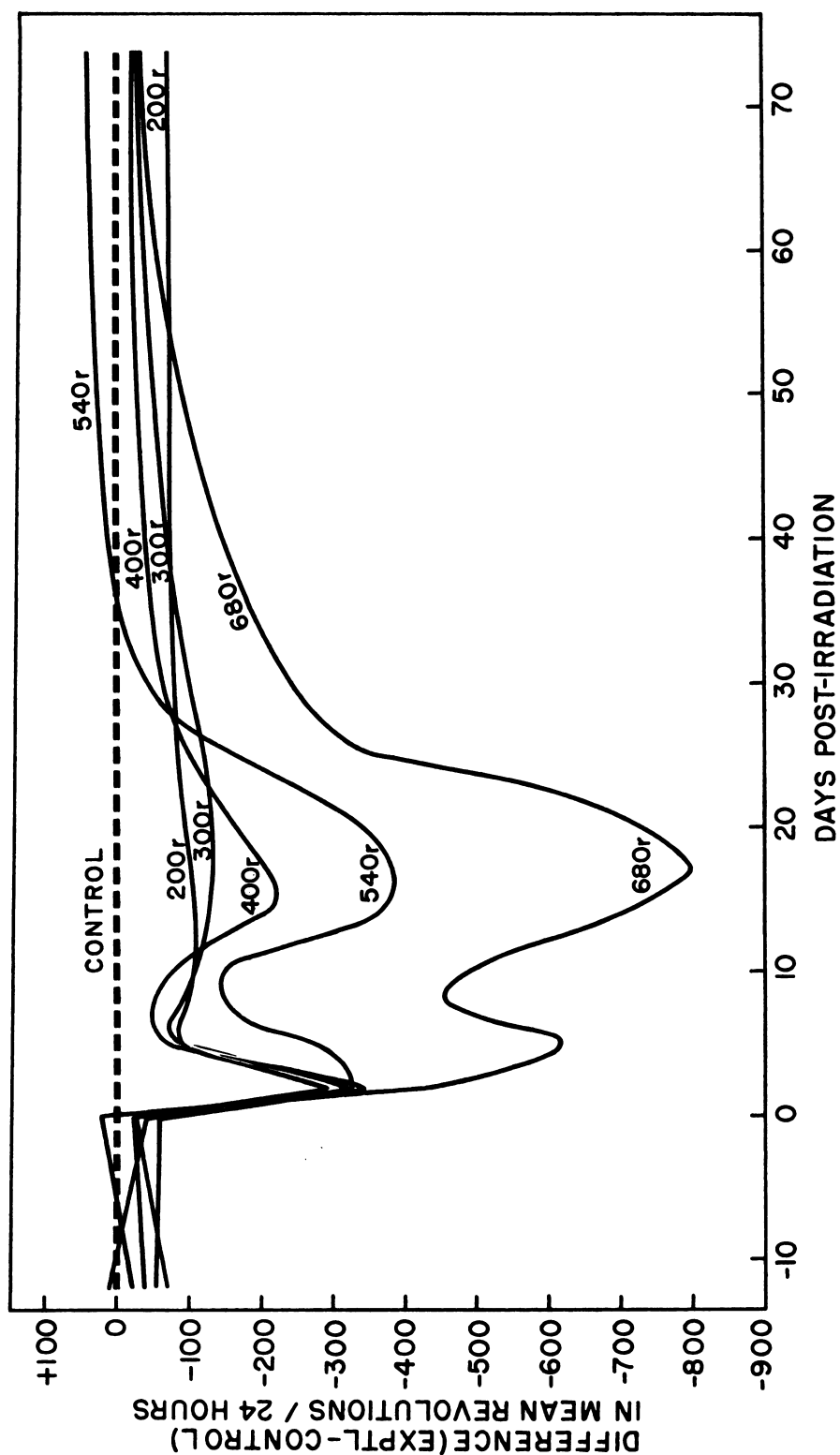


Fig. 1 The Effect of Single Doses of X Irradiation upon the Daily Volitional Activity of Surviving Male Rats

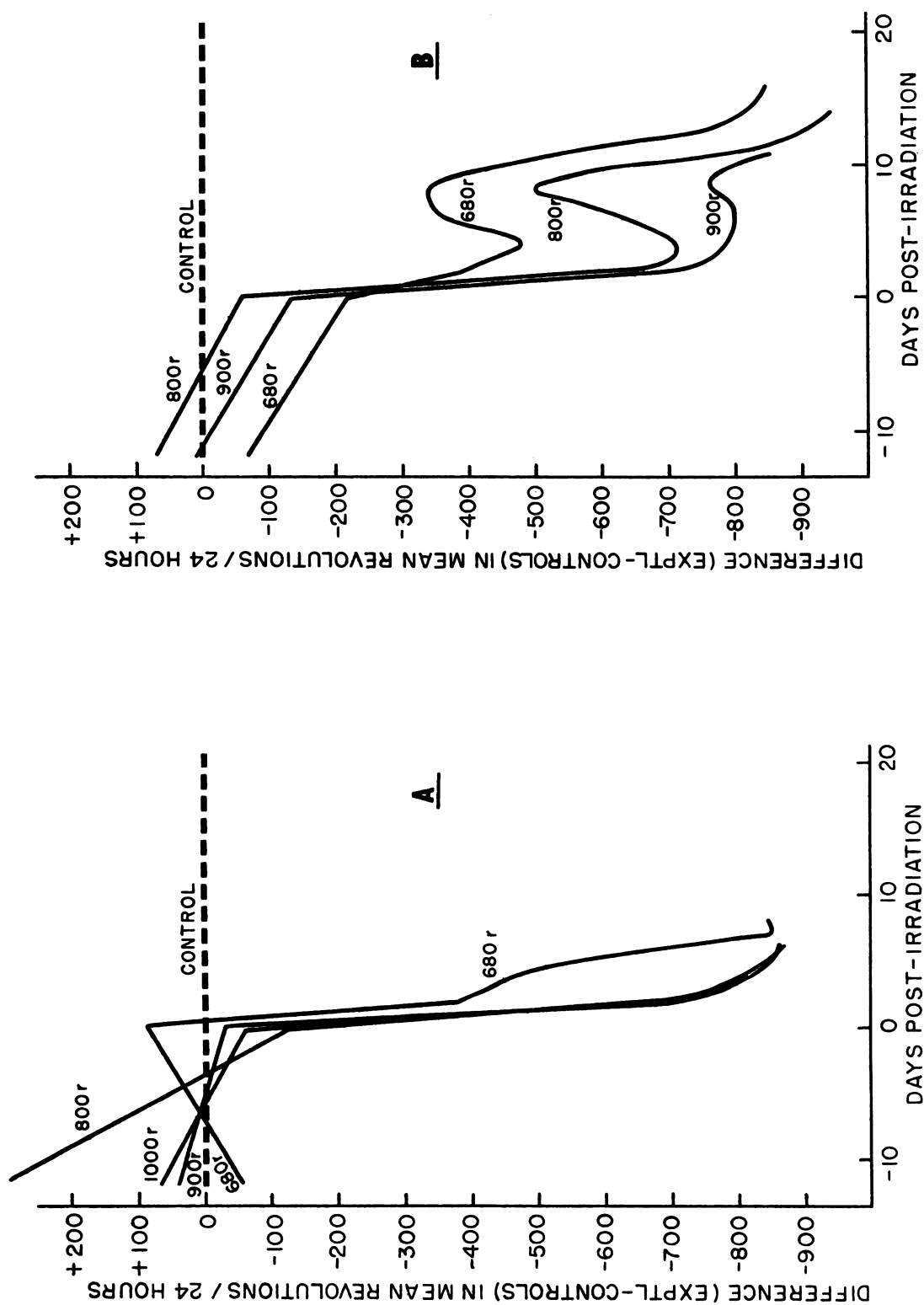


Fig. 2 The Effect of Single Doses of X Irradiation upon the Daily Volitional Activity of Nonsurviving Male Rats Which Die (a) prior to 9 Days and (b) Later Than 9 Days after Irradiation

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